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### (54) Gene and protein involved in liver regeneration

(57) Gene involved in regeneration processes of the liver and comprising a nucleotide sequence which is at least 70% homologous to the sequence of figure 1, or the complementary strand thereof, for use in the design of PCR probes for detecting nucleotide sequences in a source material, which nucleotide sequences represent genes corresponding with the gene sequence of figure 1; protein encoded by said gene for use in diagnosis of liver regeneration and/or liver cell proliferation; and antibodies directed against this protein, a PCR primer comprising at least part of said gene as a probe, and a single stranded nucleotide sequence being at least in part complementary to the messenger RNA transcribed from said gene as a probe, for use in a method for detecting the occurrence of liver cell proliferation in a subject.

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## Description

[0001] The present invention relates to the detection of a novel gene and protein involved in liver cell proliferation. The gene and protein and related molecules, such as nucleotide probes derived from the gene and antibodies directed to the protein form also part of the invention. The gene will be identified herein as RAP3 gene. The corresponding protein is called rap3 protein.

[0002] The adult liver has the capacity to regenerate after damage or partial resection. This process may allow for recovery from hepatic injuries caused by viruses, toxins, ischemia, surgery, and auxiliary liver transplantation. Liver regeneration has been studied extensively in the rat after a 70% partial hepatectomy. During the first four hours following partial hepatectomy there is a rapid, transient transcriptional activation of genes involved in the immediate early response. After induction of these immediate early genes during the transition from the quiescent state of the liver ( $G_0$ ) to the growth phase ( $G_1$ ), a delayed early gene activation is initiated which peaks during the transition of the  $G_1$  to the DNA synthesis phase (S phase).

[0003] In the research that led to the present invention novel genes involved in the delayed early response were identified by analyzing gene expression in rat liver at six hours after 70% partial hepatectomy. Upregulated genes were selected by cDNA subtractive hybridization. Upregulation was quantified by Northern blotting and the truly upregulated genes were characterized by sequence analysis.

[0004] Twelve genes were found to be upregulated at different degrees (1.5 to 10.4 fold) six hours after partial hepatectomy. Sequence analysis revealed that eight of the upregulated genes have previously been reported to be associated with liver regeneration or cell proliferation in general, one has previously been assigned an unrelated function and three have no sequence similarity to known genes.

[0005] The various upregulated genes showed two distinct gene expression patterns during a 30 hour period after partial hepatectomy. The first pattern has two peaks coincident with the  $G_1$  phases of two consecutive hepatic cell cycles. The second one shows a narrow peak at six hours after which the gene is downregulated. The novel gene which was most upregulated (3.3 fold), showed the latter gene expression pattern.

[0006] The full length cDNA of this gene was isolated from a rat liver cDNA library. Sequence analysis showed two full length cDNA's of 1282 and 1834 bp, respectively, encoding a novel protein of 367 amino acid residues. Figures 1A and 1B show the nucleotide sequence of the cDNA's. Figure 2 shows the derived amino acid sequence.

[0007] On the basis of this finding it became possible to design probes, primers and reagents for use in diagnosis.

[0008] Furthermore, based on the general 70% homology between the rat and human genome the corresponding human gene can be isolated.

[0009] Probes and primers are generally based on the nucleotide sequence of the gene. Hybridization probes can comprise the whole or a large part of the coding or complementary strand of the sequence. PCR primers are typically smaller and encompass about between 10 and 50, preferably between 15 and 30, more preferably about 20 nucleotides.

[0009] The nucleotide sequences of some suitable PCR primers are given in the following table.

Table I

primer name	nucleotide sequence
F1RAP	5' GCA TCG TGG AAA GCA TGG CT 3'
F215RAP	5' GGG ACC CTT GAG AGA GCC TG 3'
F371RAP	5' CTT GAG GCA GCA GTT GAA AC 3'
F571RAP	5' TCC ACC CTT ATG CAG AAC GC 3'
F771RAP	5' AGT ACC TTC ATC CGT GTC AG 3'
F971RAP	5' CGC CTT CGC TCC AGA GTT GG 3'
F1171RAP	5' AGG GTG GAG GGT CCT GCA TA 3'
F1371RAP	5' GCA AGC CAG TAC TTG ACC GT 3'
F1621RAP	5' GTG GTC CTG CTG GGG GAT CA 3'
R234RAP	5' CAG GCT CTC TCA AGG GTC CC 3'
R420RAP	5' CTA CCT GCT CCA TCA GCT CG 3'
R570RAP	5' AGA GTT CTT TGA CTC GGT CC 3'

Table I (continued)

primer name	nucleotide sequence
R770RAP	5' GAG CTC ATC TCG CAG CTG AT 3'
R970RAP	5' CTG TGG CTA GGC GGG GGT GG 3'
R1170RAP	5' CTG CCT ATT AGG CCA TGC TG 3'
R1370RAP	5' AGT CAG TCT CCC CCG CAC AC 3'
R1570RAP	5' TGG CAG GGA TGT ACA CAC TC 3'
R1837RAP	5' TTT CCA TCA TGA GCG TCT AT 3'

[0010] The hybridization probes can be labeled with a detectable label, such as a radioactive or biotin label.

[0011] Diagnosis of expression of the gene can be performed by means of a Northern blot. Total RNA or mRNA of a sample is separated on an agarose gel. The separation pattern is transferred to a nylon or nitrocellulose filter. An increase or decrease in the expression level is subsequently detected by hybridization with the above described hybridization probe. Typically a reference sample is included for comparison.

[0012] In case the protein is the basic macromolecule for diagnosis polyclonal or monoclonal antibodies are used for detection. The skilled person is very well capable of preparing such antibodies based on his common knowledge. Antibodies against the protein are part of the present invention.

[0013] Samples to be diagnosed can be a liver biopsy, plasma or serum. The latter can be used because the protein is secreted in the blood stream.

[0014] With the above described diagnostic methods an increase or decrease in the expression of the gene of the invention can be detected. The information that can thus be obtained is useful for establishing the efficacy of therapeutic agents stimulating liver regeneration and for patients who underwent an (auxiliary) liver transplantation and for monitoring patients treated with a bioartificial liver.

[0015] The invention is further illustrated in the following examples, which are in no way intended to be limiting to the invention. In the examples reference is made to the following figures:

30 Figure 1A is the nucleotide sequence of the 1282 bp cDNA.  
 Figure 1B is the nucleotide sequence of the 1834 bp cDNA.  
 Figure 2 shows the deduced amino acid sequence of the rap3 protein.  
 Figure 3 shows a polyacrylamide gel of liver cDNA fragments before and after subtraction. 26 cDNA fragments were found to be enriched after subtraction. Some of these are indicated by arrows. Lane 1 shows liver cDNA fragments of 6 hours 70% partial hepatectomy before subtraction. Lane 2 shows cDNA fragments of 6 hours 70% partial hepatectomy after subtraction.  
 Figure 4 shows the results of the Northern blot analysis of the temporal expression of RAP3 up to 30 hours after 70% partial hepatectomy. Panel A represents the Northern blot mRNA expression patterns at 3, 6, 12, 18, 24 and 30 hours after the 70% hepatectomy (hpx) and laparotomy (sham). Panel B represents the quantified hybridization signals indicated in PhosphorImager arbitrary units obtained at 6, 12, 18, 24 and 30 hours after the 70% hepatectomy and laparotomy.  
 The novel gene RAP3 is mostly upregulated 6 hours after partial hepatectomy after which it becomes downregulated.  
 45 Figure 5 shows a rat tissue Northern blot hybridized with a RAP3 cDNA probe. The RAP3 gene is specifically expressed in the liver.

## EXAMPLES

### 50 EXAMPLE 1

#### Isolation of RAP3 gene associated with liver regeneration

##### 1. Introduction

55 [0016] Recovery from Hepatic injuries caused by viruses, toxins, ischemia, surgery and auxiliary liver transplantation can be achieved by regeneration of the liver. The regeneration process has been studied extensively in the rat after a 70% partial hepatectomy.

[0017] During the first four hours following partial hepatectomy there is a rapid, transient transcriptional response. After this induction during the transition from the quiescent state of the liver ( $G_0$ ) to the growth phase ( $G_1$ ), a delayed early gene activation is initiated, which peaks during the transition of the  $G_1$  to the DNA synthesis phase (S phase).

[0018] This example demonstrates the isolation and identification of genes which are upregulated in the regenerating liver 6 hours after 70% partial hepatectomy.

## 2. Methods

### 2.1 Rat liver tissue preparation

[0019] Experiments were carried out in compliance with the guidelines on the care and use of laboratory animals of the University of Amsterdam. Regenerating liver was obtained from male Wistar rats (200-225 g). Rats were anesthetized with ether and subjected to midventral laparotomy. Subsequently, the left lateral and the median liver lobes were removed (70% partial hepatectomy) (G.M. Higgins and R.M. Anderson, Arch. Pathol. 12, 186 (1931)). For sham-operated animals, the liver was exposed by a midventral laparotomy.

[0020] The rats were allowed to recover from anesthesia. At 3, 6, 12, 18, 24, and 30 hours, respectively, after the 70% partial hepatectomy and sham surgery the animals were killed and the remaining liver was immediately harvested.

### 2.2 RNA isolation

[0021] Total liver RNA was isolated from liver tissue using the Trizol reagent kit (Life Technologies). Liver poly A<sup>+</sup> RNA was isolated from total liver RNA using oligo(dT)-cellulose (Boehringer Mannheim GmbH) affinity chromatography as described previously (Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular cloning: A laboratory Manual, Cold Spring Harbor, NY). To obtain highly pure poly A<sup>+</sup> RNA populations the oligo-(dT)-cellulose step was performed twice. The integrity of the poly A<sup>+</sup> RNA populations was determined on Northern blot by hybridization with glutathione-S transferase (data not shown).

### 2.3 PCR-select cDNA subtraction

[0022] The PCR-select cDNA subtraction kit (Clontech) was used to selectively amplify delayed early genes differentially expressed during liver regeneration. This method subtracts sequences common to both cDNA populations by suppressing undesirable PCR amplification, rather than by physically separating single stranded and double-stranded DNA. The 6 hours 70% partial hepatectomy liver poly A<sup>+</sup> population, containing the differentially expressed mRNA's, was compared with the 6 hours laparotomy liver mRNA population. Delayed-early genes start to appear 3 to 4 hours after the 70% partial hepatectomy. By using a laparotomy liver mRNA population rather than a normal liver mRNA population, the two populations were equalized for acute phase mRNA's, which are induced by the operation itself.

[0023] The PCR-select cDNA subtraction was performed according to the manufacturer's protocol with the following modifications. After two hybridizations, a nested PCR was used to selectively amplify the differentially expressed sequences. The second, nested PCR was performed in the presence of 0.5  $\mu$ M [ $\alpha$ -<sup>33</sup>P]dATP (1200 Ci/mmol, final volume 25  $\mu$ l). Subsequently, the amplified and differentially expressed cDNA fragments were visualized on a denaturing 4% polyacrylamide DNA sequencing gel. An X-ray film (Biomax, Kodak) was exposed overnight to the unfixed, dried gel.

[0024] Figure 3 shows the results of the subtraction. Before subtraction (lane 1), the majority of the cDNA's were poorly identifiable, indicating the presence of many cDNA fragments of different molecular size. After subtraction (lane 2), 26 distinct cDNA fragments were observed as bands that were not apparent before subtraction.

### 2.4 Isolation and identification of visualized cDNA fragments

[0025] The 26 cDNA fragments that became visible after PCR-select cDNA subtraction were excised from the dried polyacrylamide gel and heated to 100°C for 5 minutes. Subsequently, 25  $\mu$ l of the aqueous cDNA extract was used to amplify the cDNA by PCR with the nested primers used in the PCR-select cDNA subtraction. The PCR product was ligated into pCR II (Invitrogen), transformed into INVαF' competent cells, and plated out on agar plates containing ampicillin and X-Gal. Of each cloned PCR product, 6 white colonies were analyzed by PCR with T7 and SP6 primers for the presence of an insert.

[0026] Subsequently, plasmids containing an insert were purified using QIAprep (Qiagen) and the sequences of the inserts were determined using a dye terminator cycle sequencing system (Perkin Elmer) and a 377 DNA sequencer (ABI PRISM).

## 2.5 Northern blot analysis

[0027] To determine whether the expression of the genes found by the PCR-select subtractive hybridization is truly increased 6 hours after partial hepatectomy, Northern blot analysis was carried out using the purified cDNA fragments as probes. Poly A<sup>+</sup> RNA samples (0.8 µg) of the liver 6 hours after the hepatectomy and sham operation were electrophoresed on a 0.22 M formaldehyde-1% agarose gel, and blotted onto a Hybond-N nylon membrane (Amersham) by capillary transfer overnight. For fixation of the poly A<sup>+</sup> RNA the blots were baked in an oven at 80°C for 2 hours.

[0028] The inserts of the sequenced clones were amplified by PCR using the nested primers of the PCR-select cDNA subtraction method. Qiaquick-spin columns (Qiagen) were used to purify the PCR products. The purified PCR products were radioactively labelled according to the hexamer-random primed method following the manufacturer's protocol (Promega), purified on Qiaquick-spin columns (Qiagen), and hybridized with the blots. Prehybridization (2 hours, 42°C) and hybridization (overnight, 42°C) was performed in 5 x SSPE, 50% formamide, 5 x Denhardt, 0.5% SDS, and 0.1 mg/ml sheared heat-denatured herring sperm DNA.

[0029] Following hybridization the blots were washed with 2 x SSC and 0.1% SDS for 15 min at room temperature and 42°C, respectively. Subsequently, the solution was replaced with 1 x SSC and 0.1% SDS and the blots were washed for 15 min at room temperature and at 42°C, respectively. The amount of hybridization was analyzed and quantified using a PhosphorImager (Molecular Dynamics).

[0030] The fold induction of the mRNA levels observed in the 70% partially hepatectomized animals over the sham operated animals after the specific hybridization was adjusted for variability in RNA loading.

[0031] The genes which were upregulated 1.5 times or more 6 hours after 70% hepatectomy together with their identity are given in Table II. Beside these twelve genes, three genes are indicated which expression could not be detected on Northern blot. The expression of the novel RAP3 gene was found to be upregulated 3.3 fold.

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Table II

GENES UPREGULATED 6 HOURS AFTER A 70%			
	Identity of gene	Function	Fold
30	Fibronectin	Liver regeneration	1.8
	An intracisternal-A	Liver regeneration	1.8
	γ-Actin	Liver regeneration	7
35	Ribophorin I	Liver regeneration	5.5, 1.7 & 2.3
	α <sub>2</sub> -Macroglobulin	Hepatocyte proliferation <i>in vitro</i>	5.4
	Ribosomal Protein S5	Cell cycle	3.7 & 1.9
40	Ribosomal Protein L13	Cell cycle	2
	Amyloid A Protein	Growth factor	10.4
	Entactin		N.D.
	TCP-1-Containing Chaperonin related gene		1.5
45	31 kDa Putative Serine/Threonine protein kinase		N.D.
	Novel RAP1	Unknown	1.5
	Novel RAP2	Unknown	1.6
50	Novel RAP3	Unknown	3.3
	Novel RAP4	Unknown	N.D.

\* N.D. = not detectable on Northern blot

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## EXAMPLE 2

Isolation and characterization of the full length RAP3 cDNAScreening and sequence analysis

A rat liver cDNA library was prepared from poly A<sup>+</sup> RNA isolated from the rat liver 6 hours after 70% hepatectomy. To obtain full length cDNA, the Great Lengths cDNA Synthesis Kit (Clontech) was used following the manufacturer's protocol. The adaptor ligated full length cDNA inserts were cloned into the mammalian expression vector pCI at the SmaI restriction site.

After transformation into DH10B electrocompetent cells (Gibco), the cDNA library was plated at a density of 3,000 plaques per 150-mm-diameter petri dish. Colonies were lifted onto a Hybond-N nylon membrane (Amersham). The lift was hybridized with the <sup>32</sup>P-labeled RAP3 PCR fragment prepared according to the hexamer-random method following the manufacturer's protocol (Promega).

[1] Following hybridization, the lift was washed and analyzed using a PhosphorImager (Molecular Dynamics). The nine positive clones, the plasmid DNA was purified and the sequences of the inserts were determined using the nine positive clones. The plasmid DNA was purified and the sequences of the inserts were determined using the nine positive clones. The plasmid DNA was purified and the sequences of the inserts were determined using the nine positive clones. The RAP3 cDNA terminator cycle sequencing system (Perkin Elmer) and a 377 DNA sequencer (ABI PRISM). The RAP3 cDNA was obtained by comparing the nine sequences with the sequence of the RAP3 PCR fragment. Two possible clones were detected and the start and end of the cDNA were confirmed by 5'- and 3'-RACE reactions carried out following the protocol of the Marathon cDNA Amplification kit (Clontech).

[2] Based on the nucleotide sequence of the clones, PCR reactions were carried out with cDNA prepared from poly A<sup>+</sup> RNA of the rat liver 6 hours after 70% hepatectomy. The PCR products comprised the whole RAP3 cDNA, of which the nucleotide sequence was determined by bidirectionally sequencing the PCR products using 20 bp primers based on the already known nucleotide sequence data of the RAP3 cDNA.

[3] Two RAP3 cDNA molecules were detected of 1282 and 1834 bp respectively. The latter showed the same nucleotide sequence as the first, but contained an additional 552 bp nucleotide part at the 3' side.

[4] The nucleotide sequence of the 1282 bp RAP3 cDNA is as shown in Figure 1A.

[5] The nucleotide sequence of the 1834 bp RAP3 cDNA is shown in Figure 1B.

[6] Using GCG DNA software the nucleotide sequences were translated into the amino acid sequence. By analyzing the six reading frames, the largest possible protein was chosen as the RAP3 protein. Its amino acid sequence, starting with a methionine residue and ending at a stop codon, was the most likely one to form a protein in comparison to the other smaller possible proteins. Both RAP3 cDNA molecules encode the same RAP3 protein.

[7] The amino acid sequence of RAP3 protein as deduced from the nucleotide sequence is shown in Figure 2.

[8] The amino acid sequence of RAP3 protein as deduced from the nucleotide sequence is shown in Figure 2.

## EXAMPLE 3

Temporal expression between 3 and 30 hours after 70% partial hepatectomy

[9] To define the temporal expression of the RAP3 gene, mRNA levels at 3, 6, 12, 18, 24, and 30 hours after the 70% partial hepatectomy and laparotomy were analyzed by the Northern blot procedure as described in example 1. Total RNA samples (20 µg) of the rat liver isolated at the various time points were electrophoresed rather than poly A<sup>+</sup> RNA. The Northern blot was hybridized with a radioactively labeled probe comprising basepairs 370 to 1834 of the large RAP3 cDNA. The result of the Northern blot and the quantified expression pattern are given in Figure 4. The expression pattern is presented as the hybridization signal in PhosphorImager arbitrary units obtained at 3, 6, 12, 18, 24, and 30 hours after the 70% partial hepatectomy and laparotomy.

[10] Both RAP3 mRNA sizes are mostly upregulated 6 hours after partial hepatectomy after which they become downregulated.

[11] The same procedure was carried out with probes of the other upregulated genes obtained by the PCR-select subtraction. Two distinct gene expression patterns during the 30 hour period after partial hepatectomy were found. The first pattern has two peaks coincident with the G<sub>1</sub> phases of two consecutive hepatic cycles. The second one shows a narrow peak at six hours after which the gene is downregulated, just like the expression pattern of the novel RAP3 gene.

Determination of tissue specific expression

[12] A Northern blot was prepared to determine expression of RAP3 mRNA in different tissues. The various tissues (skeletal muscle, spleen, liver, kidney, heart, lung and brain) were isolated from a female Wistar rat (175 g). The experiment was carried out in compliance with the guidelines on the care and use of laboratory animals of the University of

5 Amsterdam. Total liver RNA was isolated from the tissues using the Trizol reagent kit (Life Technologies). A Northern blot was prepared from 20 µg total RNA samples and Northern blot analysis was carried out as described in example 1. A radioactively labeled probe comprising basepairs 370 to 1834 of the large RAP3 cDNA was used for the hybridization. The resulting Northern blot is given in Figure 5.

10 [0045] The RAP3 mRNA appeared to be clearly expressed in the liver and not at any detectable level in the other examined tissues. Because of this liver specificity and the 3.3 fold upregulation six hours after hepatectomy, the novel gene RAP3 was considered to be important in the process of liver regeneration.

15 **EXAMPLE 4**

20 Detection of changes of the amount of the RAP3 protein in the blood circulation

25 [0046] In order to detect changes in the amount of the RAP3 protein in the blood circulation a specific enzyme-linked immunosorbent assay (ELISA) is developed. Specific polyclonal and/or monoclonal antibodies are raised against the whole protein or a part of the protein. The protein, human or rat, is expressed in a prokaryotic or eukaryotic expression system or part of the protein is synthesized chemically. Monoclonal and polyclonal antibodies, raised in rabbits, are isolated by common techniques as described previously (Coligan, J.E., Kruisbeek, A.M., Margulies, D.M., Shevach, E.M., and Strober, W. (1994) Current Protocols in Immunology, John Wiley & Sons, Inc. Chichester, New York).

30 **EXAMPLE 5**

35 Isolation of the corresponding human gene

40 [0047] To obtain the human analogue of the RAP3 gene, a human liver cDNA library can be purchased. With this library a colony-hybridization screening is performed as described in example 2 for the detection of the rat RAP3 cDNA. Since human and rat genes have quite homologous nucleotide sequences, the rat RAP3 cDNA is used as a probe. In this way it is possible to isolate the human RAP3 gene from the cDNA library. To characterize the human RAP3 cDNA, it is sequenced as described in example 2. From the nucleotide sequence the amino acid sequence of the human RAP3 protein can be deduced.

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SEQUENCE LISTING

FORMATION:

NT:  
ME: Amsterdam Molecular Therapeutics  
STREET: Postbus 8323  
ITY: Utrecht  
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OF INVENTION: New gene and protein involved in  
liver regeneration

OF SEQUENCES: 21

TER READABLE FORM:  
MEDIUM TYPE: Floppy disk  
COMPUTER: IBM PC compatible  
OPERATING SYSTEM: PC-DOS/MS-DOS  
SOFTWARE: PatentIn Release #1.0, Version #1.30

ENT APPLICATION DATA:  
PLICATION NUMBER: EP 0 98202336.8

ON FOR SEQ ID NO: 1:

QUENCE CHARACTERISTICS:  
LENGTH: 20 base pairs  
TYPE: nucleic acid  
STRANDEDNESS: single  
TOPOLOGY: linear

ECULE TYPE: other nucleic acid

QUENCE DESCRIPTION: SEQ ID NO: 1:

AGCATGGCT

ITION FOR SEQ ID NO: 2:

QUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

OLECULE TYPE: other nucleic acid

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

10 GGGACCCTTG AGAGAGCCTG  
20

15 (2) INFORMATION FOR SEQ ID NO: 3:

10 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

25 CTTGAGGCAG CAGTTGAAAC  
20

25 (2) INFORMATION FOR SEQ ID NO: 4:

30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: other nucleic acid

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

45 TCCACCCCTTA TGCAGAACGC  
20

45 (2) INFORMATION FOR SEQ ID NO: 5:

50 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: other nucleic acid

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AGTACCTTCA TCCGTGTCAG

20

10 (2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

25 CGCCTTCGCT CCAGAGTTGG

20

30 (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: other nucleic acid

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AGGGTGGAGG GTCCTGCATA

20

45 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

55 GCAAGCCAGT ACTTGACCGT

20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: other nucleic acid

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GTGGT CCTGC TGGGGGATCA  
20

20 (2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: other nucleic acid

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CAGGCTCTCT CAAGGGTCCC  
35 20

(2) INFORMATION FOR SEQ ID NO: 11:

40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: other nucleic acid

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CTACCTGCTC CATCAGCTCG  
20

55

5 (2) INFORMATION FOR SEQ ID NO: 12:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AGAGTTCTT<sup>T</sup> GACTCGGTCC  
20

25 (2) INFORMATION FOR SEQ ID NO: 13:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: other nucleic acid

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GAGCTCATCT CGCAGCTGAT  
20

40 (2) INFORMATION FOR SEQ ID NO: 14:

40 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: other nucleic acid

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CTGTGGCTAG GCGGGGGTGG  
20

55

5 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: other nucleic acid

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CTGCCTATTA GGCCATGCTG  
20

20 (2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: other nucleic acid

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AGTCAGTCTC CCCCCCACAC  
35 20

35 (2) INFORMATION FOR SEQ ID NO: 17:

40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: other nucleic acid

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TGGCAGGGAT GTACACACTC  
20

55

## (2) INFORMATION FOR SEQ ID NO: 18:

5 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: other nucleic acid

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TTTCCATCAT GAGCGTCTAT  
 20

## (2) INFORMATION FOR SEQ ID NO: 19:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1282 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GCATCGTGGAA AAGCATGGCT GCCGTCATCA CCTGGGCACT CGCCCTCCTC TCAGTG-  
 TTTG 60

35 CAACTGTACA GGCGAGGAAG AGCTTCTGGG AGTACTTCGG CCAGAACAGC CAGGGC-  
 AAAG 120

GCATGATGGG CCAGCAGCAG AAGCTGGCAC AGGAGAGCCT GAAAGGTAGC TTGGAG-  
 CAAG 180

40 ACCTCTACAA TATGAACAAT TTCTAGAAA AGCTGGGACC CTTGAGAGAG CCTGGG-  
 AAGG 240

AGCCTCCTCG GCTGGCACAG GATCCAGAAG GCATTCGGAA GCAGTTGCAG CAAG-  
 AGCTGG 300

45 AGGAAGTGAG CACACGCCTG GAGCCCTACA TGGCTGCAAA GCACCAGCAG GTCGGC-  
 TGGA 360

ACCTGGAGGG CTTGAGGCAG CAGTTGAAAC CCTACACGGT CGAGCTGATG GAG-  
 CAGGTAG 420

50 GCCTGAGCGT GCAGGATCTG CAAGAACAGC TGCGCATGGT GGGAAAAGGC AC-  
 CAAGGCC 480

55

AGCTCCTGGG GGGCGTGGAT GAGGCGATGA GCCTGCTGCA GGATATGCAA AGTCGA-  
 GTGC 540

5 TGCACCATAAC GGACCGAGTC AAAGAACTCT TCCACCCCTTA TGCAGAACGC TTGGTG-  
 ACTG 600

GAATTGGGCA CCATGTGCAG GAGCTGCACC GGAGTGTGC TCCTCACGCA GTTGCC-  
 AGCC 660

10 CCGCGAGACT CAGTCGCTGC GTGCAGACCC TGTCCCACAA ACTCACACGT AAGGCG-  
 AAGG 720

15 ACTTGCACAC CAGCATCCAA CGCAACCTGG ATCAGCTGCG AGATGAGCTC AG-  
 TACCTTCA 780

TCCGTGTCAG CACAGACGGG GCAGACAACA GAGACTCCCT GGACCCCTCAA GCTCTC-  
 TCTG 840

20 ACGAGGTCCG CCAGAGACTC CAGGCTTTTC GACATGACAC CTACCTGCAG ATCGCT-  
 GCAT 900

TCACTCAGGC CATTGACCAG GAGACCGAGG AAATCCAGCA CCAGCTGGCA CCACCC-  
 CCGC 960

25 CTAGCCACAG CGCCTTCGCT CCAGAGTTGG GACACTCAGA CAGTAATAAG GCCCTG-  
 AGCA 1020

30 GACTGCAGAG CCGGCTGGAC GACCTCTGGG AAGATATTGC CTATGGCCTT CATGAC-  
 CAGG 1080

GCCATAGTCA GAATAACCCT GAGGGTCACT CAGGTTAACT CTGCAGCTCG TTGTCT-  
 GGAC 1140

35 CCTGAGCCTT CAGCATGGCC TAATAGGCAG AGGGTGGAGG GTCCTGCATA CTATTG-  
 GCGA 1200

GGCCACCAAA GGTGCTGCTG CCCAACCTG TCTGGCCTCC TCAACTCCCC CACT-  
 CAGGTG 1260

40 CATTACACTC AGTAGGTTTG GC  
       1282

(2) INFORMATION FOR SEQ ID NO: 20:

45 (i) SEQUENCE CHARACTERISTICS:  
       (A) LENGTH: 1834 base pairs  
       (B) TYPE: nucleic acid  
       (C) STRANDEDNESS: single  
       (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

5 GCATCGTGG AAGCATGGCT GCCGTCACTA CCTGGGCACT CGCCCTCCTC TCAGTG-  
TTTG 60

10 CAACTGTACA GCGGAGGAAG AGCTTCTGGG AGTACTTCGG CCAGAACAGC CAGGGC-  
AAAG 120

15 GCATGATGGG CCAGCAGCAG AAGCTGGCAC AGGAGAGCCT GAAAGGTAGC TTGGAG-  
CAAG 180

20 ACCTCTACAA TATGAACAAT TTCCCTAGAAA AGCTGGGACC CTTGAGAGAG CCTGGG-  
AAGG 240

25 AGCCTCCTCG GCTGGCACAG GATCCAGAAG GCATTCGGAA GCAGTTGCAG CAAG-  
AGCTGG 300

30 AGGAAGTGAG CACACGCCTG GAGCCCTACA TGGCTGCAA GCACCAGCAG GTCGGC-  
TGGA 360

35 ACCTGGAGGG CTTGAGGCAG CAGTTGAAAC CCTACACGGT CGAGCTGATG GAG-  
CAGGTAG 420

40 GCCTGAGCGT GCAGGATCTG CAAGAACAGC TGCGCATGGT GGGAAAAGGC AC-  
CAAGGCC 480

45 AGCTCCTGGG GGGCGTGGAT GAGGCGATGA GCCTGCTGCA GGATATGCAA AGTCGA-  
GTGC 540

50 TGCAACCATAAC GGACCGAGTC AAAGAACTCT TCCACCCCTTA TGCGAACGC TTGGTG-  
ACTG 600

55 GAATTGGGCA CCATGTGCAG GAGCTGCACC GGAGTGTTC GCCTCACGCA GTGCC-  
AGCC 660

60 CCGCGAGACT CAGTCGCTGC GTGCAGACCC TGTCCCACAA ACTCACACGT AAGGCG-  
AAGG 720

65 ACTTGACAC CAGCATCAA CGCAACCTGG ATCAGCTGCG AGATGAGCTC AG-  
TACCTTCA 780

70 TCCGTGTCAG CACAGACGGG GCAGACAACA GAGACTCCCT GGACCCCAA GCTCTC-  
TCTG 840

75 ACGAGGTCCG CCAGAGACTC CAGGCTTTTC GACATGACAC CTACCTGCAG ATCGCT-  
GCAT 900

80 TCACTCAGGC CATTGACCAAG GAGACCGAGG AAATCCAGCA CCAGCTGGCA CCACCC-  
CCGC 960

85 CTAGCCACAG CGCCTTCGCT CCAGAGTTGG GACACTCAGA CAGTAATAAG GCCCTG-  
AGCA 1020

90 GACTGCAGAG CGGGCTGGAC GACCTCTGGG AAGATATTGC CTATGGCCTT CATGAC-  
CAGG 1080

95 GCCATAGTCA GAATAACCCCT GAGGGTCACT CAGGTTAATC CTGCAGCTCG TTGTCT-



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

5	Met Ala Ala Val Ile Thr Trp Ala Leu Ala Leu Leu Ser Val		
	Phe Ala		
	1	5	10
15			
10	Thr Val Gln Ala Arg Lys Ser Phe Trp Glu Tyr Phe Gly Gln		
	Asn Ser		
	20	25	30
15			
	Gln Gly Lys Gly Met Met Gly Gln Gln Gln Lys Leu Ala Gln		
	Glu Ser		
	35	40	45
20			
	Leu Lys Gly Ser Leu Glu Gln Asp Leu Tyr Asn Met Asn Asn		
	Phe Leu		
	50	55	60
25			
	Glu Lys Leu Gly Pro Leu Arg Glu Pro Gly Lys Glu Pro Pro		
	Arg Leu		
	65	70	75
	80		
30			
	Ala Gln Asp Pro Glu Gly Ile Arg Lys Gln Leu Gln Gln Glu		
	Leu Glu		
	85	90	
35			
	Glu Val Ser Thr Arg Leu Glu Pro Tyr Met Ala Ala Lys His		
	Gln Gln		
	100	105	110
40			
	Val Gly Trp Asn Leu Glu Gly Leu Arg Gln Gln Leu Lys Pro		
	Tyr Thr		
	115	120	125
45			
	Val Glu Leu Met Glu Gln Val Gly Leu Ser Val Gln Asp Leu		
	Gln Glu		
	130	135	140
50			
	Gln Leu Arg Met Val Gly Lys Gly Thr Lys Ala Gln Leu Leu		
	Gly Gly		
	145	150	155
	160		
55			
	Val Asp Glu Ala Met Ser Leu Leu Gln Asp Met Gln Ser Arg		
	Val Leu		
	165	170	
	175		

His His Thr Asp Arg Val Lys Glu Leu Phe His Pro Tyr Ala  
 Glu Arg 180 185 190  
 5

Leu Val Thr Gly Ile Gly His His Val Gln Glu Leu His Arg  
 Ser Val 195 200 205  
 10

Ala Pro His Ala Val Ala Ser Pro Ala Arg Leu Ser Arg Cys  
 Val Gln 210 215 220

15 Thr Leu Ser His Lys Leu Thr Arg Lys Ala Lys Asp Leu His  
 Thr Ser 225 230 235  
 240

20 Ile Gln Arg Asn Leu Asp Gln Leu Arg Asp Glu Leu Ser Thr  
 Phe Ile 245 250 -  
 255

25 Arg Val Ser Thr Asp Gly Ala Asp Asn Arg Asp Ser Leu Asp  
 Pro Gln 260 265 270

30 Ala Leu Ser Asp Glu Val Arg Gln Arg Leu Gln Ala Phe Arg  
 His Asp 275 280 285

35 Thr Tyr Leu Gln Ile Ala Ala Phe Thr Gln Ala Ile Asp Gln  
 Glu Thr 290 295 300

40 Glu Glu Ile Gln His Gln Leu Ala Pro Pro Pro Ser His  
 Ser Ala 305 310 315  
 320

45 Phe Ala Pro Glu Leu Gly His Ser Asp Ser Asn Lys Ala Leu  
 Ser Arg 325 330  
 335

50 Leu Gln Ser Arg Leu Asp Asp Leu Trp Glu Asp Ile Ala Tyr  
 Gly Leu 340 345 350

55 His Asp Gln Gly His Ser Gln Asn Asn Pro Glu Gly His Ser  
 Gly 355 360 365

## Claims

1. Gene involved in regeneration processes of the liver and comprising a nucleotide sequence which is at least 70% homologous to the sequence shown in Fig. 1 or the complementary strand thereof.
- 5 2. Gene as claimed in claim 1, characterized in that its cDNA has a nucleotide sequence which is at least 70% homologous to the nucleotide sequence as depicted in Fig. 1 or the complementary strand thereof.
- 10 3. Gene as claimed in claims 1 and 2 for use in the design of PCR probes for detecting nucleotide sequences in a source material, which nucleotide sequences represent genes corresponding with the gene sequence shown in Fig. 1.
4. Gene as claimed in claims 1 and 2 for use as a marker of liver proliferation.
- 15 5. Protein encoded by a gene as defined in claims 1 and 2 and comprising an amino acid sequence which is at least 70% homologous to the amino acid sequence given in Fig. 2.
6. Protein as claimed in claim 5 having the amino acid sequence as depicted in Fig. 2 or the complementary strand thereof.
- 20 7. Protein as claimed in claims 5 and 6 for use in diagnosis of liver regeneration and/or liver cell proliferation.
8. Antibodies directed against a protein as claimed in claims 5 and 6.
- 25 9. Antibodies as claimed in claim 7 for use in a method for detecting the occurrence of liver cell proliferation in a subject.
10. Antibodies as claimed in claim 8 or 9 which antibodies are monoclonal antibodies.
- 30 11. Antibodies as claimed in claim 8 or 9 which antibodies are polyclonal antibodies.
12. PCR primer, comprising at least part of the gene as claimed in claim 1.
- 35 13. PCR primer, comprising at least part of the nucleotide sequence as shown in Fig. 1 or its complementary strand.
14. PCR primer as claimed in claims 12 and 13, wherein the "at least part of the nucleotide sequence" encompasses 10 to 50, preferably 15 to 30, more preferably about 20 nucleotides.
- 40 15. PCR primer as claimed in claims 12 to 14 having the nucleotide sequence as depicted in Table I or the complementary strand thereof.
16. PCR primer as claimed in claims 12 to 15 for use as a probe in a method for detecting the occurrence of liver proliferation in a subject.
- 45 17. PCR primer as claimed in claims 12 to 15 for use in the detection of gene homologous to the gene as claimed in claims 1 to 3.
18. Single stranded nucleotide sequence being at least in part complementary to the messenger RNA transcribed from a gene as claimed in claims 1 to 3.
- 50 19. Single stranded nucleotide sequence as claimed in claim 18 which is antisense RNA.
20. Single stranded nucleotide sequence being at least in part complementary to the DNA or the cDNA from a gene as claimed in claims 1 to 3.
- 55 21. Single stranded nucleotide sequence as claimed in claims 18-20, further provided with a detectable label.
22. Nucleotide sequence as claimed in claims 18 to 21 for use as a probe in a method for detecting the occurrence of

liver proliferation in a subject.

23. Nucleotide sequence as claimed in claim 22, characterized in that the method in which the nucleotide sequence is used as a probe comprises the steps of:

5  
a) obtaining a sample of a tissue or body fluid; and  
b) detecting the amount of messenger RNA transcribed from a gene as claimed in claims 1 to 3 in that sample in comparison to a reference sample by means of the probe.

10 24. Nucleotide sequence as claimed in claim 23, wherein the sample is a liver biopsy, plasma or serum.

25. Nucleotide sequence as claimed in claim 18, 20 or 21 for use as a probe for screening a liver cDNA or genomic library.

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1    GCATCGTGG AAGCATGGCT    GCCGTCA    CCTGGGCACT    CGCCCTCCTC  
 51    TCAGTGTG CAACTGTACA    GGCAGGAAAG    AGCTTCTGGG    AGTACTTCGG  
 101    CCAGAACAGC CAGGGCAAAG    GCATGATGGG    CCAGCAGCAG    AAGCTGGCAC  
 151    AGGAGAGCCT GAAAGGTAGC    TTGGAGCAAG    ACCTCTACAA    TATGAACAAAT  
 201    TTCTAGAAA AGCTGGGACC    CTTGAGAGAG    CCTGGGAAGG    AGCCTCTCG  
 251    GCTGGCACAG GATCCAGAAG    GCATTGGAA    GCAGTTGCAG    CAAGAGCTGG  
 301    AGGAAGTGAG CACACGCCTG    GAGCCCTACA    TGGCTGCAAA    GCACCAGCAG  
 351    GTCGGCTGG A CCTGGAGGG    CTTGAGGCAG    CAGTTGAAAC    CCTACACGGT  
 401    CGAGCTGATG GAGCAGGTAG    GCCTGAGCGT    GCAGGATCTG    CAAGAACAGC  
 451    TGGCATGGT GGGAAAAGGC    ACCAAGGGCC    AGCTCCTGGG    GGGCGTGGAT  
 501    GAGGGATGA GCCTGCTGCA    GGATATGCAA    AGTCGAGTGC    TGCACCATA  
 551    GGACCGAGTC A AAGAAACTCT    TCCACCCCTTA    TGCAGAACGC    TTGGTGA  
 601    GAATTGGCA CCATGTGCAG    GAGCTGCACC    GGAGTGTGTC    TCCTCACGCA  
 651    GTGCCAGCC CCGCGAGACT    CAGTCGCTGC    GTGCAGACCC    TGTCCCACAA  
 701    ACTCACACGT AAGGCGAAGG    ACTTGACAC    CAGCATCCAA    CGCAACCTGG  
 751    ATCAGCTGCG AGATGAGCTC    AGTACCTTCA    TCCCGTGTCA    CACAGACGGG  
 801    GCAGACAAACA GAGACTCCCT GGACCCCTCAA    GCTCTCTG    ACGAGGTCCG

FIG. 1A-1

851	CCAGAGACTC	CAGGCTTTTC	GACATGACAC	CTACCTGCAG	ATCCGCTGCAT
901	TCACTCAGGC	CATTGACCAG	GAGACCGAGG	AAATCCAGCA	CCAGGCTGGCA
951	CCACCCCCGC	CTAGCCACAG	CGCCTCGCT	CCAGAGTTGG	GACACTCAGA
1001	CAGTAATAAG	GCCCTGAGCA	GAUTGCAGAG	CCGGCTGGAC	GACCTCTGGG
1051	AAGATATTGC	CTATGCCCTT	CATGACCAGG	GCCATAGTCA	GAATAACCT
1101	GAGGGTCACT	CAGGTTAACT	CTGCAGCTCG	TTGTCTGGAC	CCTGAGCCCT
1151	CAGCATGGCC	TAATAGGCAG	AGGGTGGAGG	GTCCCTGCATA	CTATRTGGCGA
1201	GGCCACCAAA	GGTGGCTGCTG	CCCCAACCTG	TCTGGCCTCC	TCAACTCCCC
1251	CACTCAGGTG	CATTACACTC	AGTAGGTTTG	GC	

FIG. 1A - 2

1    GCATCGTGG AAGCATGGCT    GCCGTCA    CCTGGGCACT    CGCCCTCCCTC  
 51    TCAGTGTG CAACTGTACA    GGGGAGGAAG    AGCTTCTGGG    AGTACTTCGG  
 101    CCAGAACAGC CAGGGCAAAG    GCATGATGGG    CCAGCAGCAG    AAGCTGGCAC  
 151    AGGAGAGCCT GAAAGGTAGC    TTGGAGCAAG    ACCTCTACAA    TATGAACAAAT  
 201    TTCTAGAAA AGCTGGGACC    CTTGAGAGAG    CCTTGGGAAGG    AGCCTCTCG  
 251    GCTGGCACAG GATCCAGAAG    GCATTGGAA    GCAGTTGCAG    CAAGAGCTGG  
 301    AGGAAGTGAG CACACGCCCTG    GAGGCCCTACA    TGGCTGCAA    GCACCAGCAG  
 351    GTCGGCTGG A CCTGGAGGG    CTTGAGGCAG    CAGTTGAAAC    CCTACACGGT  
 401    CGAGCCTGATG GAGCAGGTAG    GCCTGAGCGT    GCAGGGATCTG    CAAGAACAGC  
 451    TGCGCATGGT GGGAAAAGGC    ACCAAGGCC    AGCTCCCTGGG    GGGCGTGGAT  
 501    GAGGGATGA GCCTGCTGCA    GGATATGCAA    AGTCGAGTGC    TGCACCATAC  
 551    GGACCGAGTC AAAGAACTCT    TCCACCCCTTA    TGCAGAACGC    TTGGTGACTG  
 601    GAATTGGCA CCATGTCAG    GAGCTGCACC    GGAGTGTGTC    TCCTCACGCA  
 651    GTTGCAGCC CCGCGAGACT    CAGTCGCTGC    GTGCAGACCC    GTTCCCACAA  
 701    ACTCACACGT AAGGCGAAGG    ACTTGCACAC    CAGCATCCAA    CGCAACCTGG  
 751    ATCAGCTGCG AGATGAGCTC    AGTACCTTCA    TCCGTGTCA    CACAGACGGG  
 801    GCAGACAACA GAGACTCCCT    GGACCCCTCAA    GCTCTCTG    ACGAGGTCCG

FIG. 1B-1

851	CCAGAGACTC	CAGGCTTTTC	GACATGACAC	CTACCTGGCAG	ATCGCTGGCAT
901	TCACTCAGGC	CATTGACCAG	GAGACCGAGG	AAATCCAGCA	CCAGGCTGGCA
951	CCACCCCCGC	CTAGCCACAG	GGCCTTCCGCT	CCAGAGTTGG	GACACTCAGA
1001	CAGTAATAAG	GCCCTGAGCA	GACTGAGAG	CCGGCTGGAC	GACCCTCTGGG
1051	AAGATATTGC	CTATGCCCT	CATGACCAGG	GCCATAGTCA	GAATAACCCCT
1101	GAGGGTCACT	CAGGTTAACT	CTGCAGCTCG	TGTCTGGAC	CCTGAGCCTT
1151	CAGGCATGGCC	TAATAGGCAG	ACGGTGGAGG	GTCCTGCATA	CTATTGGCGA
1201	GGCCACCAAA	GGTGCCTGCTG	CCCCAACCTG	TCTGGCCTCC	TCAACTCCCC
1251	CACTCAGGTG	CATTACACTC	AGTAGGTTTG	GCAAACACAG	CTTCGGGTGC
1301	TCATTTGGGA	TCCTAAGGAG	CAAGAGTGGG	GTGAAGGGAG	TGGGGAGATG
1351	GTGTGGGGG	GAGACTGACT	GCAAGCCAGT	ACTTGACCGT	TGCTAGAAC
1401	CTGTGTCACT	ACAAACCTGGA	GGCCGGCTCC	TATTACCTCA	TGCCCTGATGC
1451	TCGCTGTTAT	AGTCGGTCTA	CAGAGGGAA	CTCCCTGTCTC	CCCAGGGTTG
1501	TCATGACAGC	CTTTGTTGGA	AGAGAGCAGG	AGAACATGAC	ACGTATGATG
1551	GAGTGTGTAC	ATCCCCTGCCA	GTGGTCCTGC	TGGGGAAATC	AGTGATGGGA
1601	TAATGTGTG	CATCCCCTGCA	GTGGTCCTGC	TGGGGATCA	GTGATGGGAT
1651	GGGCAGAGC	CCCTTATTCCC	TTAGAGAACT	CTAACCCAAA	TAAGGAACCTG

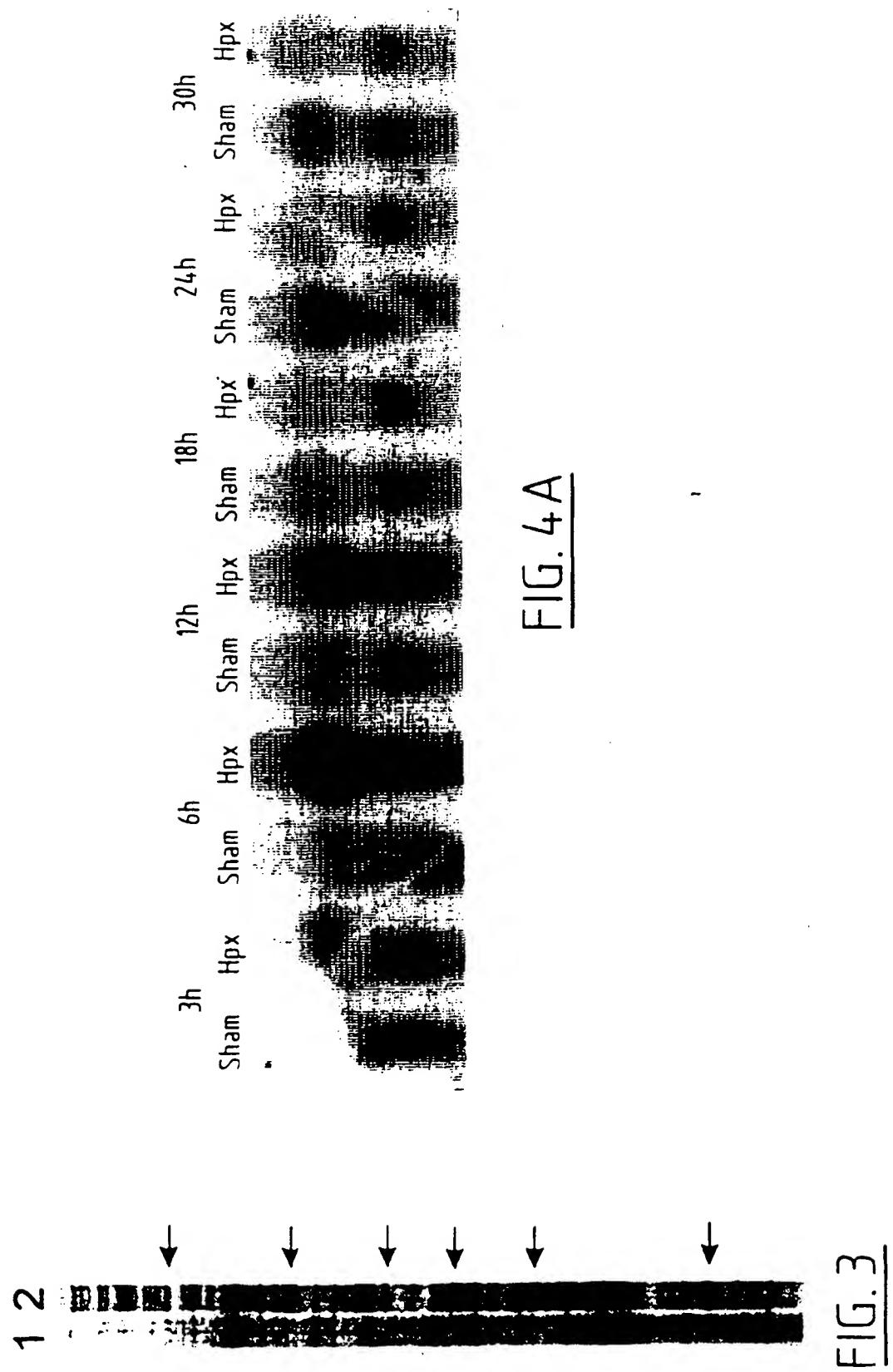
FIG. 1B-2

1701 AGCCCTCTGC ACTGAGGGCT TCTGAAACC CTGTACATAG CAAACTGTGT  
 1751 GCCCTCTTCA TCATGCAGTC CCCACCTCCT GATTCTCGGG ATGGAACCTGA  
 1801 CTTTTGGTTG GAATGAAATA GACCGCTCATG ATGG

FIG. 1B-3

1 MAAVITWALA LLSVFATVQA RKSEWEYFGQ NSQGKGMMGQ QQKLAQESIK  
 51 GSLEQDLYNM NNFLKEKLGPL REPGEPPRL AQDPEGIRKQ LQQELEEVST  
 101 RLEPYMAAKH QQVGVWNLEGL RQQQLKPYTVE LMEQVGLSVQ DLQEQLRMVG  
 151 KGTKAQLLGG VDEAMSLLQD MQSRVLHHTD RVKELFHPYA ERLVTGIGHH  
 201 VQEILHRSVAP HAVASPARLS RCVQTLSHKL TRKAKDLHTS IQRNLDQLRD  
 251 ELSTFIRVST DGADNRDSL D PQALSDEVRQ RLQAFRHDTY LQIAAFTQAI  
 301 DQETEEIQHQ LAPPPPSHSA FAPELGHSDS NKALSRLQSR LDDLWEDIAY  
 351 GLHDQGHSON NPEGHSG\*

FIG. 2



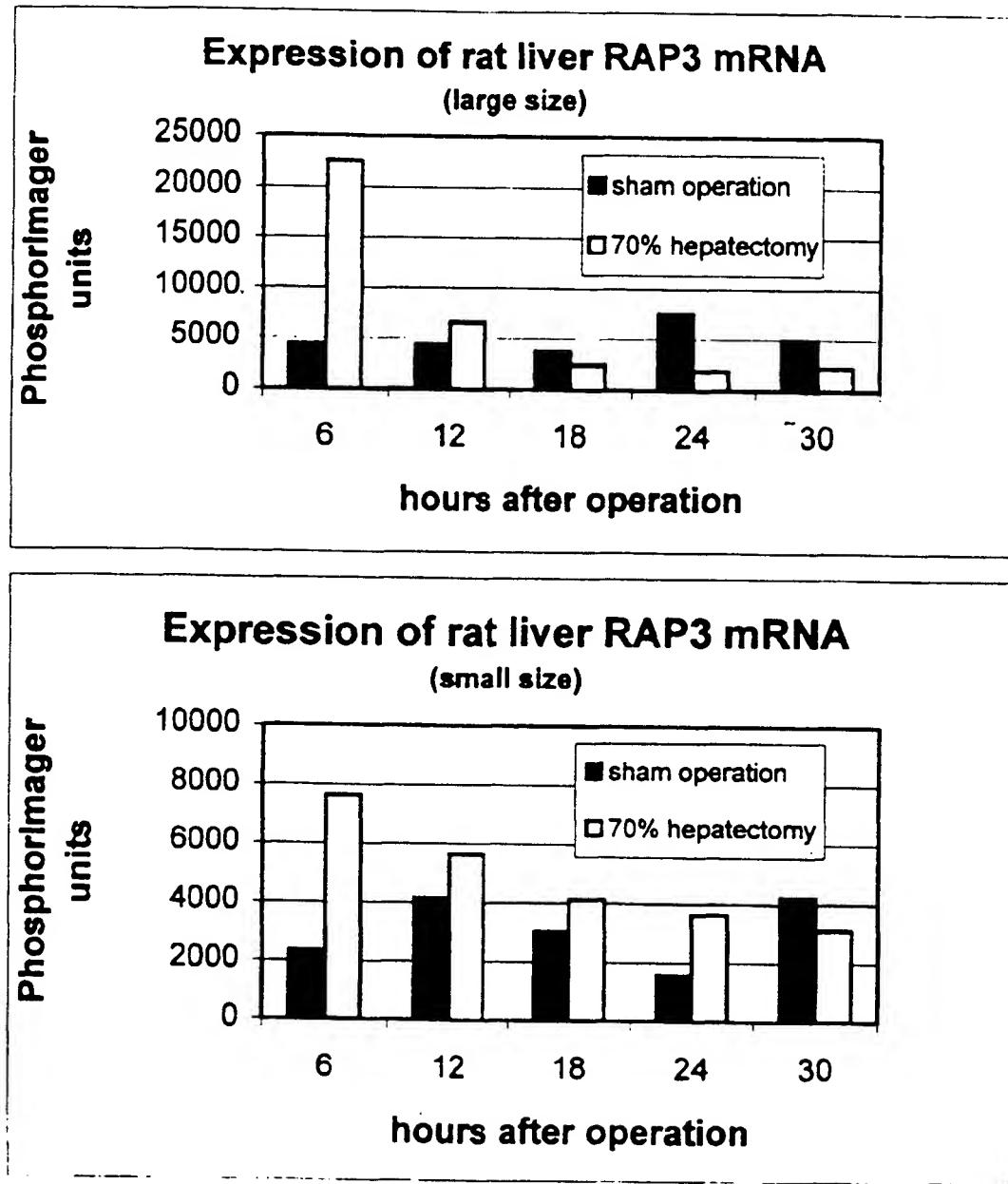


FIG. 4B

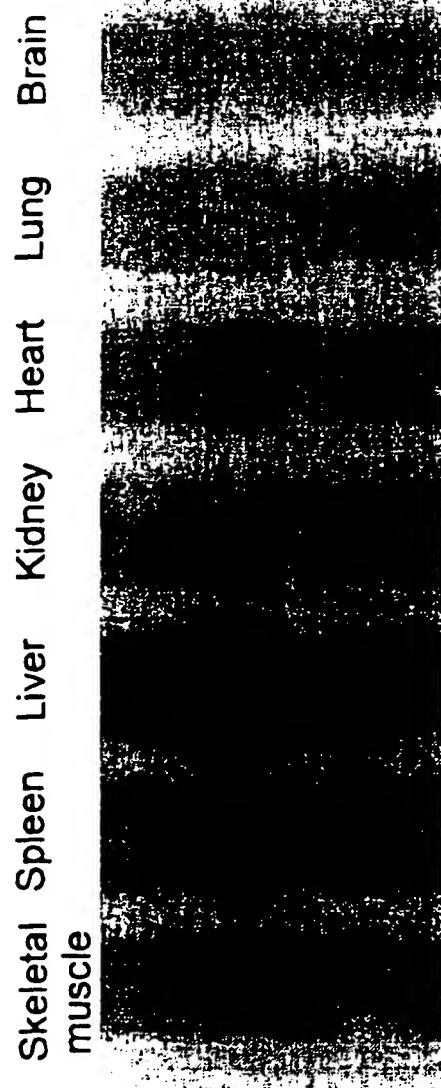


FIG. 5



European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number

EP 98 20 2336

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (BNCI6)						
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim							
X	MARRA M ET AL.: "Sugano mouse liver mRNA Mus musculus cDNA clone 1431407 similar to apolipoprotein A-IV (accession number AA987093)" EMBL SEQUENCE DATABASE, 29 May 1998, XP002095461 Heidelberg, Germany * the whole document *	3,12-14, 16-25	C12N15/12 C07K14/47 C07K16/18 C12Q1/68						
A	WO 96 39540 A (ADVANCED TISSUE SCIENCES INC) 12 December 1996 * the whole document *	1-25	-						
			TECHNICAL FIELDS SEARCHED (BNCI6)						
			C07K C12N C12Q						
<p>The present search report has been drawn up for all claims</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Place of search</td> <td style="width: 33%;">Date of completion of the search</td> <td style="width: 33%;">Examiner</td> </tr> <tr> <td>THE HAGUE</td> <td>3 March 1999</td> <td>Oderwald, H</td> </tr> </table>				Place of search	Date of completion of the search	Examiner	THE HAGUE	3 March 1999	Oderwald, H
Place of search	Date of completion of the search	Examiner							
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<p>Category of Cited Documents</p> <table border="0" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; vertical-align: top;">           X : particularly relevant if taken alone            Y : particularly relevant if combined with another document of the same category            A : technological background            D : non-written disclosure            P : Intermediate document         </td> <td style="width: 50%; vertical-align: top;">           T : theory or principle underlying the invention            E : earlier patent document, but published on, or after the filing date            D : document cited in the application            L : document cited for other reasons            I : member of the same patent family, corresponding document         </td> </tr> </table>				X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background D : non-written disclosure P : Intermediate document	T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons I : member of the same patent family, corresponding document				
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ON EUROPEAN PATENT APPLICATION NO.

EP 98 20 2336

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03-03-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639540 A	12-12-1996	AU 6160396 A CA 2223707 A EP 0832289 A	24-12-1996 12-12-1996 01-04-1998

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82